Supplemental Experimental Procedures

Serum immunoglobulin isotype quantification

Serum immunoglobulin isotypes were measured with Beadlyte mouse immunoglobulin isotyping kit from Millipore (St. Charles, MO) according to manufacturer's recommendations. This was used to quantify IgG_1, IgM, IgA, IgG_3, IgG_{2a} and IgG_{2b} from serum samples of mice between 45 and 160 days old. Measurements and calculations of standard curves were made using the Bio-Plex 200 instrument and software from Bio-Rad (Hercules, CA).

Real-time RT-PCR primer sets

HPRT: CTTCCTCCTCAGACCGCTTT & ACCTGGTTCATCATCGCTAA,

Eomes: CCCCTATGGCTCAAATTCC & GGGGTTGAGTCCGTTTATGTT,

Tbet: AGTTCAACCAGCACCAGACA & GAAGGACAGGAATGGAACA,

$S1P_1$: GTGTAGACCCAGAGTCCTGCG & AGCTTTTCCTTGGCTGGAGAG,

KLF2: AGCCTATCTTGCCGTCCTTT & CGCCTCGGGTTCATTTC,

IL-4: GAGACTCTTTCGGGCTTTTC & TGATGCTCTTTAGGCTTTCCA,

CCR1: TCAAAGGCCCAGAAACAAAG & GCTGAGGAACTGGTCAGGAA,

CCR3: GCAGGTGACTGAGGTGATTG & GTGGAAAAAGAGCCGAAGGT,

CCR5: AGGAGGTGAGACATCCGTTC & AACTGACCCTTGAAAATCCATC,

CCR6: ATCCTGGGTCTTTCGGACTT & GGCAGTTCAACCACACTCTC,

CCR7: CAGCCTTCCTGTGTGATTTCTACA & ACCACCAGCACGTTTTTCCT,

CXCR1: CCAACAGGCAGGCTTTAGTT & CAGCATCACCAGCGAGTTT,

CXCR2: GCCACTCAGAGAACCTGGAA & ACCAAGGAGTTCCCCACAAG,

CXCR3: AGCCAAGCCATGTACCTTGA & CTCGTTTTCCCCATAATCG,

CXCR5: GGAGACCCCCATAAAGGAAA & ACACCGAGGAGGAAGATGAG,

CXCR6: GCCTGGCTTCTCTTGCATCT & CAGGCTCTTCAGCTTCTGGT,

CXCR7: AGTGTCCCACCATGCCTAAC & TATTCACCCAGACCACCACA,

XCR1: GCACTGGAGGAGATCAAAGG & ATCTGGACGCGGGATG

Figure S1. Nonconventional T cells are overrepresented in the CD4 gate of KLF2 KO mice. Iterative gating strategy of thymus, spleen, and liver from left to right: all lymphocytes, CD4 single positive, CD25- and TCRγ-negative, NK1.1- and $CD1^d$/αGal-cer tetramer-negative. Numbers indicate the percentage of relevant gate. Numbers to the right indicate percentage of the respective CD4 SP gate that is TCRβ+, and CD25-, TCRγ-, NK1.1-, CD1d-tet-. The increase in proportion of nonconventional T cells in periphery is a result of the dramatic decrease in conventional T cells not an increase in nonconventional T cells. For example, in the spleen there was a 25-fold decrease in conventional T cell number and a 1.4-fold decrease in nonconventional T cell number comparing KLF2 KOs to WT mice. Results are representative of 4 mice in each group.

Figure S2. A predominance of KLF2 KO thymocytes is necessary for CXCR3 expression on KLF2 KO cells. Flow cytometry for surface expression of CXCR3 on "dump negative" SP thymocytes from the indicated mixed bone marrow chimeras. Matching colors indicate the same animal. Black= 50% WT:50% WT control, red= 10% WT: 90% KLF2 KO, and green= 90% WT: 10% KLF2 KO. Results are representative of greater than 5 independent chimera setups with 1-3 mice/group in each.

Figure S3. A KLF2-GFP fusion knock-in mouse faithfully reports KLF2 expression. A) Strategy used to engineer the $KLF2^{GFP}$ knock-in mouse. B) Number of TCRβ+, "dump negative" CD4 and CD8 splenocytes from age-matched wild type (WT), CD4-cre x KLF2 flox (KO), and $KLF2^{GFP}$ knock-in (reporter) mice. C-G) Flow cytometric analysis of thymus from homozygous $KLF2^{GFP}$ mice. C) Left panel shows the CD4 by

CD8 profile of all thymocytes. The middle panel shows GFP expression on total thymocytes, black line= $KLF2^{GFP}$ reporter and shaded gray= WT control. Right panel shows the CD4 by CD8 profile of GFP+ thymocytes from $KLF2^{GFP}$ mice. D) GFP expression in the indicated thymocyte subset. Numbers indicate percent GFP+ cells in that subset. E) TCRβ and TCRγ expression in the GFP+ DN gate. F) Left panel shows gating for semi-mature and mature CD4SP. Histogram overlays (right) show GFP expression of semi-mature (gray) and mature (black) CD4 SP thymocytes from $KLF2^{GFP}$ mice. G) Histogram overlays show $S1P_1$ cell surface expression of GFP- (filled gray) and GFP+ (black line) in CD4 SP thymocytes. Results are representative of at least 2 mice.

Figure S4. CXCR3 upregulation in the KLF2 deficient thymus occurs in the absence of CD1-selected thymocytes A) Top panel: CD4 by CD8 profile of CD4-cre/$KLF2^{fl/fl}$ and CD4-cre/$KLF2^{fl/fl}$/$CD1^{-/-}$. Bottom panel: $CD1^{d}$-αGalCer by TCRβ profile of all thymocytes. B) Histogram overlays of flow cytometric analysis of the IL-4 receptor α and CXCR3 on gated TCRβ+, "dump negative" CD4 SP and CD8 SP thymocyte populations, red line= CD4-cre/$KLF2^{fl/fl}$, blue line= CD4-cre/$KLF2^{fl/fl}$/$CD1^{-/-}$ and shaded gray= KLF2 WT control. Results are representative of at least 2 mice per group and independent experiments.

Figure S5. Limited overexpression of other Ig isotypes in mice with KLF2 deficient T cells. Serum IgG_1, IgM, IgA, IgG_3, IgG_{2a} and IgG_{2b} levels. Serum was collected from mice 45-160 days old. WT: N=6, KLF2 KO: N=10.

Figure S6. IL-13R KO cells are susceptible to the bystander effect. Flow cytometry of CXCR3 expression on "dump negative" SP thymocytes from the indicated mixed chimeras. Percentages indicate the proportion in "dump negative" CD4SP gate. Results are representative of 3 mice in each group.

Figure S7. Model of KLF2 cell-autonomous and cell-nonautonomous effects. A) KLF2 deficient thymocytes lack CD62L and $S1P_1$ and are retained in the thymus. B) KLF2 deficient thymocytes spontaneously produce IL-4. C) IL-4 signaling through the type I IL-4 receptor and dependent on the transcription factor eomesodermin leads to the upregulation of the chemokine receptor CXCR3 on both WT and KLF2 KO (not depicted) thymocytes.